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ENDONUCLEASE IN CULTURE SUPERNATANTS AND CELL-FREE EXTRACTS OF E. COLI K12 STRAINS

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U.S. ARMY BIOLOGICAL LABORATORIES
Fort Detrick, Frederick, Maryland

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ENDONUCLEASE IN CULTURE SUPERNATANTS AND CELL-FREE EXTRACTS OF *E. COLI* K12 STRAINS

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ABSTRACT

The deoxyribonuclease activities of culture supernatant fluids and crude sonic extracts of various substrains of Escherichia coli are reported. The assay methods consisted of measuring the destruction of transforming Bacillus licheniformis DNA, and the appearance of acid-soluble materials absorbing ultraviolet light at 260 mμ.

Culture supernatants of F^+ and Hfr strains in most cases inactivated transforming DNA to a greater extent than supernatants of F^- strains. Sonic extracts also destroyed transforming DNA and no difference in activity was noted among F^-, F^+, and Hfr strains. Acid-soluble materials absorbing ultraviolet light at 260 mμ increased slowly when all extracts were incubated with calf thymus DNA. Calf thymus DNA protected transforming DNA from destruction in the presence of cell-free extracts.

The rapid destruction of transforming DNA by culture supernatants and cell-free extracts and the slow appearance of acid-soluble materials suggest that extracellular and intracellular endonuclease activity is responsible for the observed effects.

ENDONUCLEASE IN CULTURE SUPERNATANTS AND CELL-FREE EXTRACTS OF *E. COLI* K12 STRAINS

Extracts of *Escherichia coli* B possess several exonucleases and an endonuclease that have been extensively studied by Richardson et al.[1] and by Lehman et al.[2] In addition, a new phage-related exonuclease has been found by Korn and Weissbach,[3] in association with the induction of λ phage development in *E. coli* K12.

The recent survey by Rothberg and Swartz,[4] for extracellular DNAase in members of the Enterobacteriaceae failed to produce evidence of extracellular activity among 182 strains of *E. coli* tested, using the agar plate method for determining hydrolytic activity on DNA.

Our experiments report the deoxyribonuclease activities of culture supernatants and crude sonic extracts of various substrains of *E. coli* K12. The major assay method measured the destruction of transforming *Bacillus licheniformis* DNA by such preparations. Other methods studied the effect of sonic extracts on calf thymus deoxyribonucleic acid, using the increase in acid-soluble materials absorbing ultraviolet light at 260 mμ as the indicator of activity, and the protective effect of calf thymus deoxyribonucleic acid on transforming DNA in biological assay.

Table 1 describes the effect of culture supernatants of various strains on transforming *B. licheniformis* DNA. *E. coli* strains were incubated overnight in L broth of Luria and Burrous,[5] then diluted 1:10 in fresh L broth and incubated 3 hours with shaking at 37 C. At that time, 2-ml amounts were incubated at 37 C with 32 μg of *B. licheniformis* 9945A transforming DNA for 3 hours. The cultures were then centrifuged and the supernatants were removed, heated to 65 C for 30 minutes to kill remaining viable cells, and stored overnight at 4 C. The supernatant-DNA mixtures were diluted 1:10 by adding to competent cells of *B. licheniformis* 9945A, which require glycine and histidine. The transformation technique of Thorne and Stull[6] was followed.

Table 1 records the per cent of transformants arising on minimal agar supplemented with glycine after exposure to DNA that had been incubated with the *E. coli* supernatants as compared with controls with untreated DNA.

The strains have been grouped according to the state of the fertility factors. Notice that the effect of the supernatants on the transforming DNA causes a vast reduction in the number of transformants, and that the degree of reduction depends in most cases on the presence or absence of the fertility factor. The notable exceptions are strains AB-359 (F^-) and Hfr C. The presence or absence of the fertility factor in these two strains was reconfirmed by mating experiments.

TABLE 1. DESTRUCTION OF TRANSFORMING ACTIVITY OF B. LICHENIFORMIS DNA BY CULTURE SUPERNATANTS OF E. COLI STRAINS[a]

Fertility State	Strain	Per Cent Survival[b]
F^-	AB-6	6.7
	M-54	8.4
	AB-334	14.0
	AB-359	0.4
	L-12 (K3)	100.0
	W-1177	68.0
F^+	W-1485	0.05
	M-65	0.004
	L-23	0.012
Hfr	M-23-Hfr H	0.002
	AB-311	0.04
	Hfr C	10.0
	AB-312	1.2
	M-12-Hfr 4	0.022
	P4X6	0.028

a. Incubation time, 3 hours.
b. Compared with untreated DNA controls.

Since this experiment measures the extracellular DNAase activity of culture supernatants of K12 substrains, it appears that strains possessing the fertility factor, in either the Hfr or F^+ states, discharge more enzyme or a more active enzyme into the medium.

Table 2 describes the intracellular DNAase activities of the same strains. Extracts were prepared from cultures that were incubated 6 hours in the absence of transforming DNA. The cells were washed once with 0.01 M phosphate buffer, pH 7.0, were resuspended in buffer to one-fourth or one-tenth of the original volume, and were then broken during 12- to 20-minute treatments in a 10-kc Raytheon sonic oscillator. The cell debris was passed through a sintered glass filter and stored at 4 C until used. The activity of the extracts did not decrease during storage periods of several weeks.

TABLE 2. DESTRUCTION OF TRANSFORMING ACTIVITY OF B. LICHENIFORMIS DNA BY CELL-FREE EXTRACTS OF E. COLI STRAINS

Fertility State	Strain	Per Cent Survival[a] after Incubation for 0.5 hr	1.5 hr	2.5 hr
F⁻	AB-6	0.6	0.4	0
	M-54	1.3	0.02	0
	AB-334	0.04	0.0015	0
	AB-259	0.6	0	0
	L-12 (K3)	1.4	0.84	0.14
	W-1177	0.8	0	0
F⁺	W-1485	0.48	0.04	0
	M-65	0.7	0	0
	L-23	0.35	0	0
Hfr	M-23-Hfr H	0	0	0
	AB-311	0.25	0.02	0
	Hfr C	3.4	2.5	0.7
	AB-312	0.033	0	0
	M-12-Hfr 4	0.4	0.06	0
	M-18-P4X6	0.2	0	0
	Hfr 4-F2	0.13	0	0

a. Compared with untreated DNA controls.

For the assay, one ml of extract was incubated with shaking at 37 C with 16 μg of transforming DNA and assessed periodically for remaining transforming activity. The mixtures of extract and DNA were then diluted 1:10 by addition to competent cells, and the transformation procedure was followed. Protein concentration of the extracts was determined by the method of Lowry et al.[7] and varied from 2.07 to 9.92 mg/ml.

The data on Table 2 record the inactivation of transforming DNA during incubation with the extracts. The results show that intracellular DNAases are present in all the strains tested and that the degree of destruction of transforming DNA is not related to the presence or absence of the fertility factor. The extract of strain L-12 (F⁻) was the least destructive, which was also true in the experiment involving supernatants.

Table 3 describes an experiment that approaches the problem differently. The degradation of calf thymus DNA by the sonic extracts forming products soluble in cold 0.5 N perchloric acid was measured. For the assay, the sonic extracts were diluted 1:6 and were incubated with 200 μg/ml of calf thymus DNA in the presence of 0.01 M phosphate buffer, pH 7, and 0.02 M $MgSO_4$ for up to 24 hours. They were then diluted 1:20 in buffer, an equal volume of 1 N perchloric acid was added, and the mixtures were placed at 4 C for 15 minutes. This treatment was followed by centrifugation, and the optical densities of the supernatants were determined at 260 mμ in a Beckman DU spectrophotometer. The procedure was a modification of that used by Richardson et al.,[1] for the autolysis of cell extracts.

TABLE 3. RELEASE OF COLD PERCHLORIC ACID SOLUBLE MATERIAL AFTER INCUBATION OF CALF THYMUS DNA WITH E. COLI EXTRACTS FOR 24 HOURS

Fertility State	Strain	Δ OD Extract + DNA - Δ OD Extract Alone
F^-	AB-6	0.155
	M-54	0.171
	AB-334	0.075
	AB 359	0.085
	L-12 (K3)	0.042
	W-1177	0.106
F^+	W-1485	0.090
	M-65	0.083
	L-23	0.047
Hfr	M-23-Hfr H	0.004
	AB-311	0.056
	Hfr C	0.075
	AB-312	0.055
	M-12-Hfr 4	0.056
	M-18-P4X6	0.073
	Hfr 4-F2	0.078

The differences between the control and extracts incubated with DNA indicate a definite increase in acid-soluble products when the extracts were incubated with DNA. The formation of acid-soluble products was very slow in the extracts, requiring 20 to 24 hours incubation under aseptic conditions. The increase did not seem to be dependent on the presence or absence of the fertility factor in the strains. Strain AB-6 (F-) showed one of the greatest increases in acid-soluble products, which occurred on three different occasions, and might indicate that this strain produces more enzyme than the other strains.

The assay just described involved sonic extracts diluted 1:6, whereas the transformation experiments employed undiluted extracts. For a few experiments, extracts have been diluted as much as 1:100 and then incubated with transforming DNA. Such an experiment is described next.

The experiment described here reflects the protection of transforming DNA by calf thymus DNA in the presence of a sonic extract of strain W-1485 (F+). The extract was diluted 1:100 with 0.01 M phosphate buffer, and then incubated for various periods with 40 or 80 μg/ml of calf thymus DNA and 6 μg/ml of transforming DNA. At intervals the mixtures were further diluted 1:10 with competent cells and the transformation procedure was followed. Figure 1 illustrates that calf thymus DNA is able to protect transforming DNA from the rapid destruction illustrated by the control curve, and adds more evidence to our assumption that destruction of transforming DNA is due to DNAase activity. Classical competition between the two kinds of DNA was evident in controls containing no extract. The control transformation rates were reduced as a function of the concentration of calf thymus DNA; however, the competitive effect was clearly distinguishable from the inhibition of destruction of transforming activity by the DNAase.

Although transforming DNA is rapidly reduced by cell products and sonic extracts of K12 substrains, the appearance of perchloric acid-soluble products in extracts incubated with calf thymus DNA is extremely slow. These observations suggest that endonuclease activity is responsible for both results. The activity of transforming DNA can be rapidly destroyed by breaks in the centers of polynucleotide chains, which is indicative of endonuclease activity. In fact, Richardson et al.,[3] have used assays of the biological activity of B. subtilis DNA as an indicator of endonuclease activity, and have mentioned that such an assay is a very sensitive test for endonuclease.

The inability to detect extracellular DNAase activity in the strains of E. coli tested by Rothberg and Swartz[4] can probably be explained by our results. Their assay depends on the rapid cleavage of DNA to small acid-soluble products that result from exonuclease action. Our evidence points to a potent extracellular endonuclease activity that would not readily be detected by this method. The differences in extracellular nuclease activity between F- and F+ or Hfr strains are intriguing. It is conceivable that the markedly increased elaboration of DNAase by males precludes efficient mating between F+ cells and confines transfer of small red genome to the male plus female conjugal pairs.

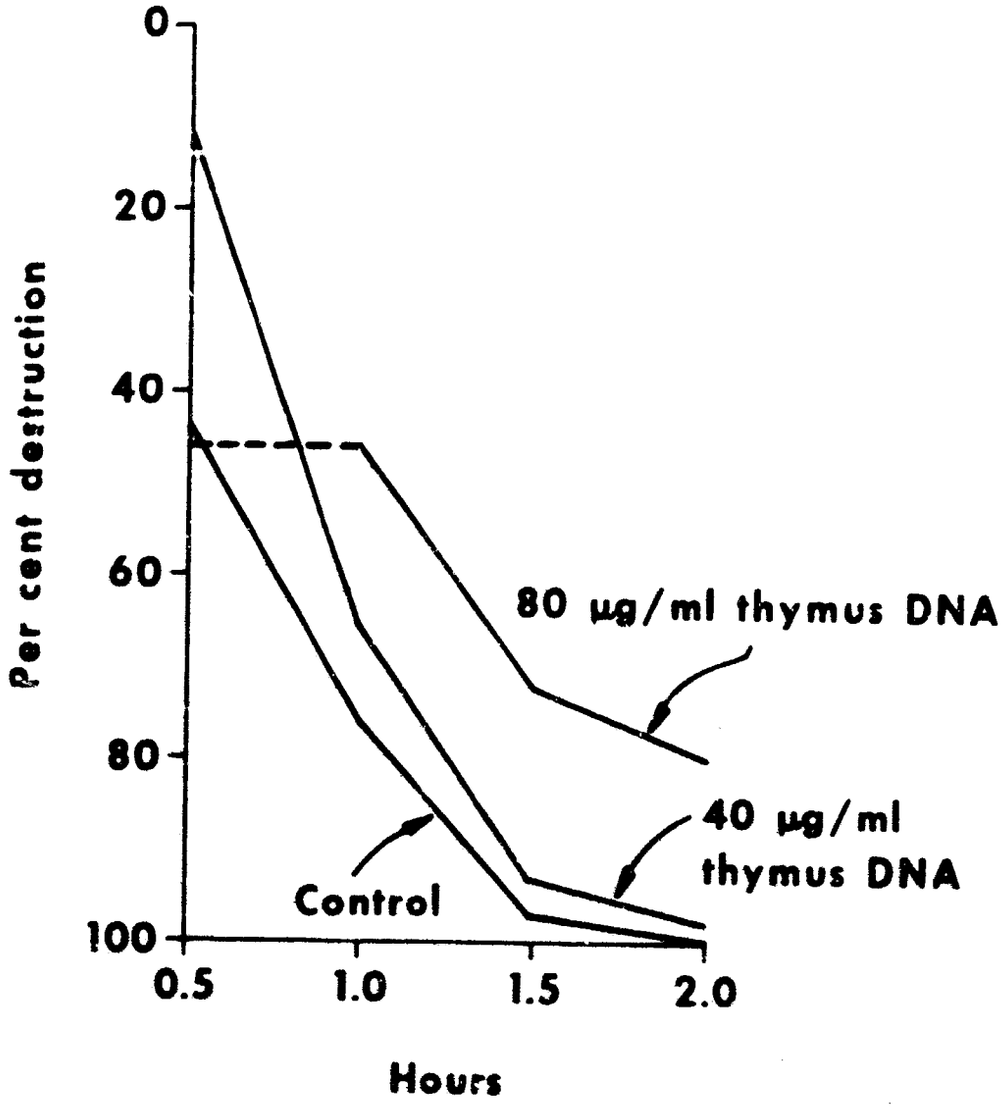

Figure 1. Destruction of B. licheniformis DNA in presence of Calf Thymus DNA by Sonic Extract of E. coli W-1485.

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